THE DIGESTIVE SYSTEM AND ITS FUNCTION IN *FUNDULUS HETEROCLITUS.*

B. P. BABKIN AND D. J. BOWIE.

(From the Atlantic Biological Station, St. Andrews, N. B. and Department of Physiology, University of Toronto.)

In the course of investigation of the function of the alimentary canal in *Fundulus heteroclitus* (North American common killifish, mudfish, mummichog [1]), it was found that this animal does not possess a stomach, *i.e.*, an organ secreting the pepsin-hydrochloric acid. Although several species of fishes do not possess peptic glands (see in Oppel's "Lehrbuch der Vergleichenden Mikroskopischen Anatomie der Wirbeltiere," 1 Teil, Jena 1896, S. 33, the corresponding table), we have never seen any reference to any investigation concerning the gastric digestion in *Fundulus heteroclitus.*[1] From the anatomical as well as from the physiological point of view, the digestive system of the *Fundulus* presents great peculiarities, which are worth attention. The present paper is to be regarded as a preliminary communication. A series of investigations were made by one of us (B. P. B.) during the summer of 1926 at the Atlantic Biological Station, St. Andrews, N. B., and subsequently one of us (D. J. B.) made histological examinations at the Department of Physiology, University of Toronto.

Anatomical Data.

The whole family of Pœciliidæ are comparatively small fishes. The largest specimens of *Fundulus* taken at Birch Cove on the Bay of Fundy, near St. Andrews, N. B., were from 65 to 83 mm.

[1] It is interesting to note that the whole family of Cyprinidæ (*Cyprinus carpio,* etc.) are deprived of peptic glands. *Fundulus* belongs to the family of Cyprinodontidæ, and the Germans call it "Amerikanische Zahnkarpfen" (*Brunning* (2)). According to *Gill* (3) "the Cyprinodonts or Poecilids are really related to the Esocids and Umbrids, and to them they should be approximated in the sub-order Haplomi." The results of the present investigation, especially the absence of the glands secreting the pepsin-hydrochloric acid, show that *Fundulus* has some features in common with the Cyprinidæ.

in length, measured from the snout to the insertion of the caudal fin.

The alimentary canal in this species is comparatively short, being about equal to the length of the body, excluding the caudal fin. On opening the abdomen and carefully dissecting away the liver, we see a short œsophagus directly connected to the intestine.

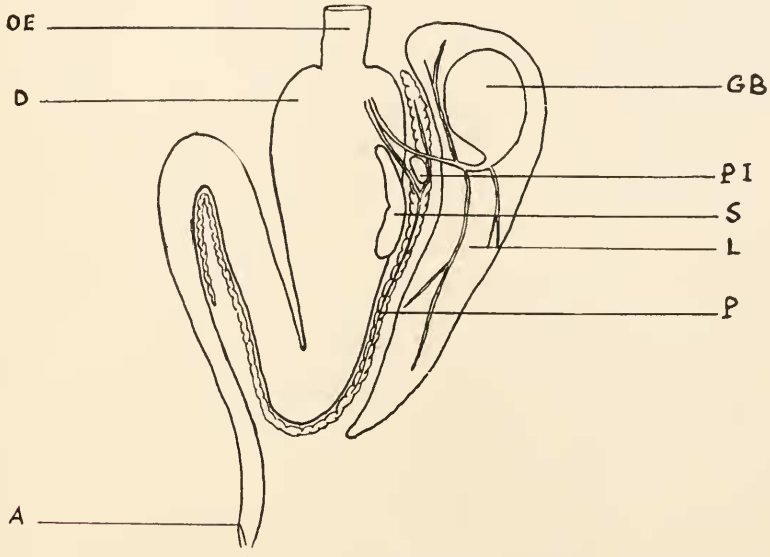

FIG. 1. Diagram of the alimentary tract of *Fundulus*. *Œ*, œsophagus; *D*, duodenum; *GB*, gall-bladder; *P.I.*, principal islands of Langerhans; *S*, spleen; *L*, liver; *P*, pancreas; *A*, anus.

Longitudinal sections through the junction of œsophagus and intestine show that, at this point, there is a well-developed sphincter quite comparable to the pyloric sphincter in mammals. The intestine is bent upon itself ventrally and to the right to form three portions; a first portion, descending; a second portion, ascending; and a third portion, descending to the rectum (Fig. 1). Pyloric cæcæ are not present.

The first part of the intestine, especially when distended with food, has a somewhat pear-shaped form. The second and third parts of the intestine do not display any such unusual degree of dilatation when food is present in them.

In Fig. 2 is represented a scheme of the alimentary tract of

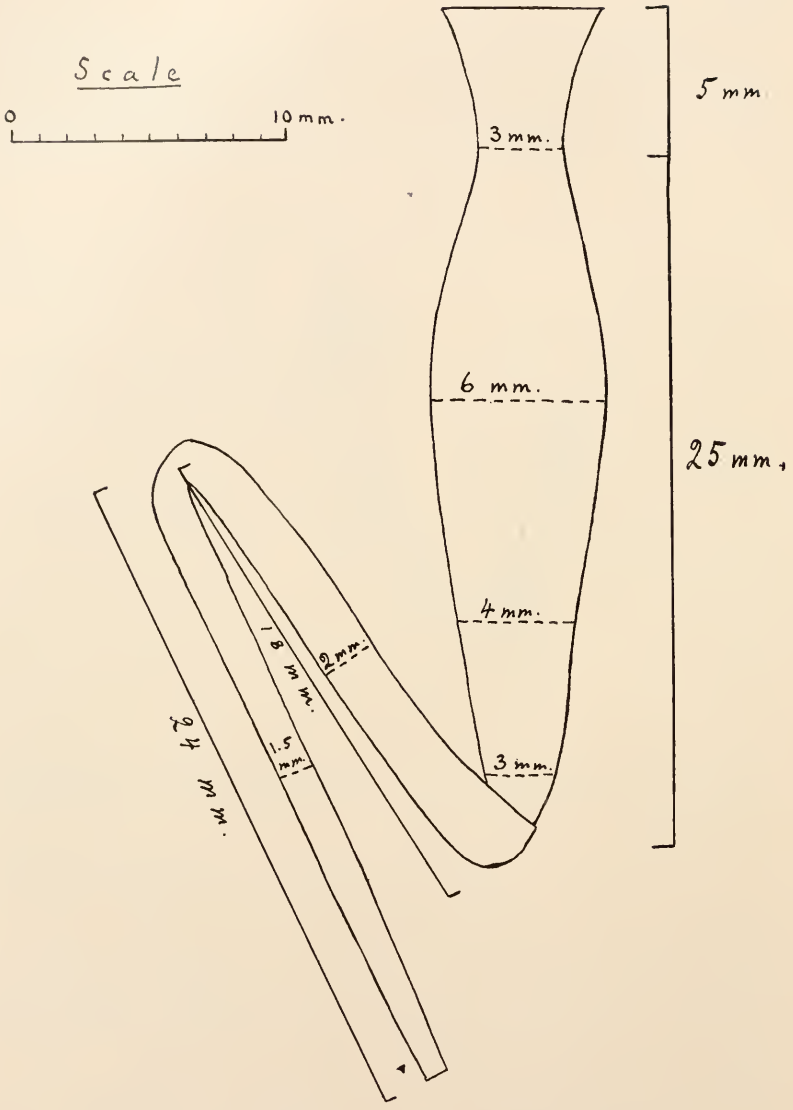

FIG. 2. Scheme of the alimentary tract of *Fundulus*. The intestinal tract is fully relaxed.

Fundulus. The actual measurements are taken from a female *Fundulus* whose body length (caudal fin excluded) was equal to 78 mm.

The capacity of the first part of the intestine is four times greater than the capacity of the second part. Without pressure in the first part, four drops of water enter; in the second part only one drop enters. Under a moderate pressure (filling from a pipette) the cardiac part retains 7 drops, and 8 drops more may be introduced into it by using a greater pressure. Thus the arrangement of the initial part of the alimentary canal of *Fundulus* is similar to the so-called "syphonal" stomach of many other fishes. From a physiological point of view the first part of the intestine of the *Fundulus* fulfils one of the duties of the stomach, being *a receptacle and container for the food.*

In order to avoid repetition of cumbersome phraseology we shall frequently designate the first part of the intestine as the duodenum, since, as will be shown, it is into this part that the bile and pancreatic juice are poured. Both the physiological and the anatomical observations show that a stomach is really absent in *Fundulus*.

The liver is comparatively large, as it is in most fishes, and lies in a left ventral position, closely applied to the œsophagus and to the loop formed by the first and second portions of the intestine. In the upper part of the longitudinal space bounded by the liver and the three portions of the intestine, we find the gall-bladder, the spleen and the "principal" islets of Langerhans, along with a considerable portion of the diffuse pancreas. The pancreas has a wide distribution along the three parts of the intestine and is so diffuse that in fresh specimens it cannot readily be differentiated with the naked eye. The gall-bladder is comparatively large in these fish; in a specimen 78 mm. in body-length it measured 6 mm. by 3.5 mm., and had a capacity of 2.5 drops. The duct which drains the gall-bladder arises from the posterior end of that vesicle, but soon turns in an anterior direction and, after passing along the first part of the intestine to within 3 or 4 mm. of the œsophagus, it pierces the intestinal wall.

It is important to note that the pancreatic duct, though it runs parallel to the bile duct, does not unite with the latter to form a common duct, but has its own separate opening into the intestine

beside the orifice of the bile channel. The hepatic ducts communicate with the cystic duct to form the common bile duct, though one or more of them may empty directly into the neck of the gall-bladder. The spleen is a small, flattened, bright red organ, and serves as a convenient landmark for locating the so-called principal islets of Langerhans, which are situated in the region between the neck of the gall-bladder and the duodenum. The largest of them, the "chief" principal islet, appears as a whitish, spherical nodule about 1 mm. in diameter. The pancreas is best studied in a series of stained sections. These show that slender processes extend off from the larger portions of the gland to invade the liver, particularly along the blood vessels and less so along the hepatic ducts. Sections show also that a thin layer of pancreatic tissue completely envelopes the gall-bladder. Serial sections made with the aim to study the duct connections of the pancreas which surrounds the gall-bladder showed that the ducts are leading away from this pancreatic tissue and communicating with the larger pancreatic ducts. It is important to note, however, that in no case were we able to find any evidence of communication between any of the channels for bile and any of those for pancreatic juice.

Methods.

Since the fistula method is not practical for the study of the digestive processes in fishes, after a suitable interval following natural or forced feeding the contents of the duodenum were sucked out by means of a pipette with a rubber bulb on the end. This method had the well known disadvantage that the intestinal secretions were mixed with food masses and also that removal of the partially digested food caused more or less disturbance of the normal process of digestion. *Funduli* are exceptionally hardy fishes and did not show any ill effects from the manipulations connected with the investigations of their intestinal contents.

The fish were kept in small aquaria or in glass jars containing running sea-water, or else in jars in which the water was kept cool (below 15° C.) and was well aërated. When kept in non-aërated sea-water which is allowed to reach a temperature of 15° to 20° C. (a comparatively high temperature for these *Funduli*), the fish very often eject the food through the mouth and some of them may die under such unfavorable conditions.

The reaction of the intestinal contents was first tested by means of litmus paper. Since the indications of litmus are not very reliable, whenever possible the hydrogen ion concentration was determined by the "spot" method elaborated by Felton (4). The values of the pH found in the present investigation must be looked upon as approximate only, because in most cases the intestinal fluids were mixed with food masses, a fact which would introduce the "protein" and "salt" errors described by Clark (5). The presence of bile in the intestinal contents was determined by Gmelin's test and by the surface tension test with flowers of sulphur. The methods employed for the detection of different enzymatic actions will be given later on.

As a preliminary measure all of the fish were deprived of food for one or more days prior to the experiments.

Experiments on Fasting *Funduli*.

Usually in fishes which have fasted for one or more days the duodenum is practically empty, it being difficult to obtain even as much as one drop of fluid in the pipette and what is obtained is composed partly of mucous. In some cases one may obtain two or three drops of a yellowish fluid which gives quite positive results in tests for bile, and occasionally the remains of copepods and other small animals may be found when microscopic examination of the contents is made. After 7 to 10 days of starvation, it is hard to get anything at all from the duodenum. Water is very seldom present in this part in fasting *Fundulus*.

The contents of the duodenum of fasting *Fundulus* is always alkaline to litmus, and its hydrogen ion concentration, as determined in more than 50 fishes ranges from pH 8.0 to pH 9.2. The variations in alkalinity depend chiefly on the presence or absence of bile, since bile taken directly from the gall-bladder has a hydrogen ion concentration of only pH 7.0 to 7.2. When mucus alone was present, the values were pH = 8.8 to pH = 9.2, whereas when bile also was present the values recorded were from pH = 8.0 to pH = 8.6. Regarding the possible presence and influence of sea water, we may say that samples taken from the sea-water pipe system at St. Andrews Biological Station were found in several determinations to have pH values of from 8.0 to 8.1. The con-

tents of the second and third parts of the intestine of freshly killed fasting fish were also always alkaline to litmus.

Experiments on Fed *Funduli.*

Although *Funduli* prefer animal food such as clam and the internal organs of dead fishes, even of their own species, they are practically omnivorous and will eat fish muscles, raw meat, bread,

Table I.

Digestion of Clam in *Fundulus.*

Date.	Fish No.	Contents of Intestine.	Reaction of the Contents.	
			pH.	Litmus.
July 17:	1	Yellowish fluid	8.8	Strong alkaline
9.30 A.M.	2	" "	8.6	" "
to	3	" mucus	8.8	" "
10.00 A.M.	4	Mucus and water	8.6	Alkaline
	5	Mucus	8.8	Strong alkaline
10.15 A.M.		12 pieces of clam were put in the aquarium; 6 pieces were eaten at once. At 12.00 noon two pieces were left, which were removed from the aquarium		
12.15 P.M.	3	Pieces of clam; no bile	8.4	Alkaline
	4	Scant; no bile	8.8	Strong alkaline
2.15 P.M.	1	Clam; bile	8.2	Alkaline
	2	" "	8.8	"
	3	Scant; "	8.6	"
	4	Nil.	8.6	"
	5	Clam; bile	8.4	"
7.15 P.M.	1	Nil.	8.4	"
	2	Clam; bile	8.2	"
	3	Mucus only	8.8	Strong alkaline
	4	" "	9.0	" "
	5	Clam; a little bile	8.8	" "
July 18:				
9.00 A.M.	5	Yellowish mucus	9.2	" "

hardboiled egg (yolk and white) and even watermelon. They digested perfectly well milk introduced by a pipette into the duodenum. The alimentary tract of freshly caught *Funduli* may contain mud, and small animals, insects, copepods, etc., but no matter what might be the nature of the food eaten or the stage of its

digestion, the reaction in the duodenum as well as in the second and third parts of the intestine was always alkaline, both in fish that were caught and investigated at once, on the spot, and also in those kept in aquaria and fed naturally or forcibly. However, the degree of alkalinity varied somewhat, according to the nature of the contents. As stated above, the reaction in the duodenum of fasting *Funduli* is decidedly alkaline, pH = 8.0 to 9.2. The presence of foods rich in protein, such as meat and clam, which are acid; of milk, which is neutral or only very slightly alkaline; of sea-water (pH = 8.2); and particularly the presence of considerable amounts of bile, which is also nearly neutral (pH = 7.0 to 7.2), all tend to reduce the degree of alkalinity.

Table I shows the course of digestion in *Fundulus* after natural feeding with clam. The contents of the duodenum were sucked out at certain intervals for each of five fishes. Prior to feeding they had been fasting for five days, and no excrements were found in the aquarium during the last two days of this period. Shortly before the fast was terminated, the pipette was introduced into the duodenum to withdraw any fluids which might be present.

Results analogous to those shown in the table were obtained with all the other kinds of food; the reaction in the duodenum was always alkaline.

The following experiment shows that there is an active discharge of bile into the intestine after a meal has been swallowed.

Experiment of August 4.

In an aquarium in which nine fishes were kept for several days without food, at 8.30 A.M. there were placed several pieces of clams' flesh, but the amount was not sufficient to feed all of them. At 8.30 P.M. all of these fishes were killed and the contents of the duodenum and of the gall-bladder were investigated. In five fishes the duodenum was filled with clam and bile, while the gall-bladder was quite empty and collapsed. In the other four fishes the duodenum was empty, while the bladder was distended with bile.

In the case of injection of milk into the duodenum, the digestion was also always alkaline, the degree being somewhat less in the first hours (pH = 7.8) than in the later hours (pH = 8.8). Bile was present in the contents. The pH of the milk itself was pH = 7.2.

Alkaline reaction was also always found during the digestion of raw beef introduced into the duodenum. Six and one half hours after forcible feeding the contents were sucked out and the reddish-yellow fluid obtained gave an alkaline reaction, pH = 8.2.

Reaction to Various Stimuli.

Mechanical Stimulation.

Experiment of July 10.—As a means of mechanical stimulation, a few small pieces (2 mm. cubes) of cork or rubber were introduced into the duodenum in three *Funduli,* which were starved for at least three days. Just prior to the experiment the contents of the duodenum, consisting of a very little amount of mucus and some bile, were tested and found to be alkaline, pH = 8.6 to 9.0.

At 10.15 A.M., three pieces of cork were introduced into the duodenum of one fish; two pieces into that of the second; and two pieces of rubber into that of the third fish. The duodenal contents were sucked out at 2.15 P.M. and at 6.15 P.M. At both times, in each case, one could remove from 2 to 4 drops of water, with some mucus, but in only one fish was bile present, as indicated by the slightly yellowish colour of the contents. The reaction was in all cases alkaline, pH = 8.2 to 8.6. The next morning the corks were found in the aquarium, stuck together with mucus and probably had been ejected through the anal opening.

It is interesting to note that towards the end of the experiment water was found in the duodenum of these fishes, since its presence is quite unusual. From these experiments it appears that purely mechanical stimulation of the duodenum does not provide the necessary stimulus to provoke the pouring of bile into the intestine but that it does cause the fish to "drink water."

Hydrochloric Acid.

Table II, gives the results of introducing a few drops of 0.36 per cent. hydrochloric acid into the duodenum in two fishes that had been starved for 10 days. Its introduction did not provoke a flow of bile into the gut.

The table shows that in 6 hours after the introduction of 0.36 per cent. hydrochloric acid the duodenal contents had again become alkaline. It is interesting to note that in warm blooded animals the introduction of hydrochloric acid in the concentration in which

TABLE II.

INTRODUCTION OF HCl INTO DUODENUM.

Date and Time.	Fish No.	Contents of Intestine.	pH.	Litmus.
August 13:				
9.00 A.M.	1	Almost nil.	—	Alkaline
	2	" "	—	"
9.10 A.M.		Introduced 5 to 6 drops 0.36% HCl		
10.10 A.M.	1	Mucus and fluid	4.8	Acid
	2	" " "	5.0	"
11.10 A.M.	1	Mucus; very little fluid [1]	5.0	"
	2	" " " " [1]	5.4	"
12.10 P.M.	1	Mucus only [1]	5.0	"
	2	" " [1]	5.4	"
3.10 P.M.	1	Mucus and water	7.8	Very weak alkaline
	2	Watery fluid	7.6	" " "

it is in the gastric juice, *i.e.*, 0.5 to 0.36 per cent. practically does not provoke the entry of bile into the duodenum.

Alcohol.

From 4 to 6 drops of 5 per cent. pure ethyl-alcohol was introduced into the duodenum by means of a pipette 3 to 4 times per day. Four sets of analogous experiments were performed. In one of them, 2 fishes received alcohol regularly during 6 days. In the earlier experiments, in order to make sure that the alcohol reached the duodenum, it was mixed with a little finely ground carmin and in every case the red granules were afterwards found in the duodenal contents and also in the excrements.

The results of one of these experiments are shown in Table III. The fish had been starved for 2 or 3 days before the experiment, and the duodenum was almost empty. The very small amount of fluid present was alkaline to litmus. When the duodenum was evacuated 2 or more hours after injection of alcohol, it was found to contain only a very small amount of fluid, usually mucus. Except occasionally in the morning tests after a night

[1] To determine the reaction in these cases a few drops of distilled water were added to extracted material.

when the fish did not receive injections of alcohol, there was no bile present in the duodenum. The alkalinity of the contents was very high, pH = 8.8 to 9.2, but it was not determined whether this was due to the presence of pancreatic juice or of intestinal juice. The alcohol injected was slightly acid, pH = 6.8.

TABLE III.

INTRODUCTION OF ALCOHOL INTO THE DUODENUM.

Date and Time.	Contents of Intestine.	pH.	Litmus.	5% Alcohol Injected.
July 19:				
4.30 P.M.		—	—	4 drops
7.00 P.M.	1 drop of fluid	8.6	Alkaline	" "
July 20:				
9.30 A.M.	Mucus	9.0	"	" "
2.45 P.M.	2 drops	8.8	Strongly alkaline	" "
7.00 P.M.	2 drops	8.8	Alkaline	" "
July 21:				
11.25 A.M.	1 drop and mucus	8.2	Alkaline	" "
12.25 P.M.	3 drops	8.8	Strongly alkaline	" "
3.30 P.M.	2 drops	9.0	" "	" "
7.15 P.M.	2 drops	9.0	" "	None
July 22:				
11.50 A.M.	Mucus only [1]	9.4	" "	4 drops
July 23:				
9.45 A.M.	Nil.	—	—	" "
11.50 A.M.	3 drops	8.8	Strongly alkaline	" "
2.45 P.M.	1 drop and mucus	9.0	" "	" "
July 24:				
9.15 A.M.	Mucus only [1]	9.2	" "	" "
12.15 P.M.	1 to 2 drops	9.2	" "	None

N. B. Bile was not found in the contents during this experiment.

It is important to note that the experiments with alcohol afford further evidence that gastric glands are absent in Fundulus. Although alcohol is one of the strongest stimuli for the secretion of gastric juices, in our experiments the intestinal contents never had an acid reaction, but, as stated above, were always highly alkaline.

Pilocarpin.

Table IV. gives the results of one of eight sets of experiments with pilocarpin. Five or 6 drops of 1 per cent. solution of pilocarpin was injected into the duodenum of two fishes which had

[1] To determine the reaction in these cases a few drops of distilled water was added.

been starved for 7 days. At the end of this period the duodenum contained in each case a small amount of mucus and fluid which was strongly alkaline, pH = 9.2 and 9.0 respectively.

The interesting feature of the experiments is that pilocarpin provoked chiefly the motor phenomena of the alimentary canal. Its introduction into the duodenum excited evacuation of the bowel and this was accompanied by the entry of the bile into the intestine. The contents of the duodenum, extracted one hour after injection of the pilocarpin, had a yellowish or greenish colour and gave a positive test for bile, with flowers of sulphur. Following the injection, the alkalinity of contents of the duodenum was comparatively low, pH between 7.8 and 8.6.

TABLE IV.

INTRODUCTION OF PILOCARPIN INTO THE DUODENUM.

Date and Time.	Contents of Intestine.	pH.	Litmus.
August 10:			
8.35 A.M.	1. Mucus and some fluid	9.2	Alkaline
	2. " " " "	9.0	"
8.45 A.M.	Injected 5 to 6 drops of 1% sol. of pilcarpin into each fish		
9.45 A.M.	1. 2 to 3 drops of emerald green bile	8.0	Weakly alkaline
	2. 8 drops of green fluid; re-injected 6 drops of it	7.8	" "
10.45 A.M.	1. 2 to 3 drops of light green fluid	8.4	" "
	2. 10 drops of green fluid; re-injected 8 drops of it	8.2	" "
11.45 A.M.	1. 2 to 3 drops of light green fluid	8.4	Alkaline
	2. 8 drops of green fluid	8.2	Weakly alkaline
3.15 P.M.	1. 4 to 5 drops of a very light green fluid with mucus	8.6	Alkaline
	2. 3 to 4 drops of clear, colorless fluid	8.6	"

Atropin.

The injection of 1 per cent. atropin sulphate into the duodenum, as one would expect, inhibited the intestinal secretions (it was hard to get anything but mucus from the intestine) and also prevented the entry of bile into the gut. Table V. gives the results of experiments on two fishes which had been starved for 10 days.

The reaction of the duodenal contents was usually strongly alkaline.

It is interesting to note that one hour after the introduction of atropin into the intestine, the skin of the fish became dark, almost black. In five hours the normal colour of the skin had returned. These changes were not due to light effects, as the whole day was grey and cloudy.

From the foregoing experiments it may be seen that the first part of the alimentary canal of Fundulus may properly be designated the duodenum, since it corresponds to the duodenum of vertebrates possessing a stomach.

TABLE V.

INTRODUCTION OF ATROPIN INTO THE DUODENUM.

Date and Time.	Contents of Intestine.	pH.	Litmus.
August 13:			
9.10 A.M.	1. Nil.	—	Alkaline
	2. "	—	"
9.20 A.M.	Injected 4 to 5 drops of 1% sol. of atropin sulphate into each fish		
10.20 A.M.	1. 2 to 3 drops of fluid, with mucus	9.0	Strongly alkaline
	2. 1 drop of fluid, with mucus	8.8	Alkaline
11.20 A.M.	1. Very little fluid and mucus; difficult to obtain it	8.8	"
	2. Very little fluid and mucus; difficult to obtain it	8.8	"
12.20 P.M.	1. Almost nil.; a little mucus	8.8	Strongly alkaline
	2. Almost nil.; a little mucus	8.8	Alkaline
3.20 P.M.	1. Mucus and 1 to 2 drops of water	9.0	Strongly alkaline
	2. Mucus and 3 drops of water	8.8	" "

N. B. There was no bile present in the intestinal contents during the whole experiment.

ENZYMES OF THE MUCOUS MEMBRANE OF THE FIRST PART OF THE INTESTINE.

The only method which we applied to the study of the enzymes of this mucous membrane was the reaction of its extract on various media. The imperfections of this method are very well known. A certain possibility that we might have to consider autolytic enzymes, as well as digestive enzymes, was suggested by the ob-

servations of Bradley (1922[6]), on autolysis of liver and kidney. He produced evidence to show that in autolysis there are two autolytic enzyme-complexes, one of which is analogous to trypsin, but has its optimum activity at pH 4 to 4.5, whereas true trypsin has its optimum activity at pH 8. Beyond the range from pH 7 to pH 3, the action of this autolytic enzyme-complex is insignificant, while for trypsin the range of activity is pH 9 to pH 4.5. The other autolytic enzyme-complex is analogous to pepsin, but has its optimum reaction at pH 4.5, whereas pepsin has its optimum at pH 1.5. It is destroyed at pH 2.6, while pepsin is very active at that degree of acidity. The range of activity for pepsin is from pH 0.5 to pH 6.5 (McFarlane, et al.[7]).

By adjusting the reaction of the media to hydrogen ion concentrations which are beyond the limits of activity for any autolytic enzyme-complexes which might be present, one could expect to obtain reactions due solely to digestive enzymes. To minimize the possibilities of contamination all extracts of the intestinal mucous membrane were kept under toluene and all test tubes and pipettes were sterilized before use.

The extracts from the mucous membrane usually of the duodenum were prepared in the following way. The intestine, cut out from the body, was cleaned from all surrounding tissues, split longitudinally, and washed several times in tap water. Only the mucous membrane was scraped by a knife and mixed with the corresponding fluid. The temperature in the incubator was 35° C.

For use in the various experiments extracts of the mucous membrane of the intestine were made with 30 per cent. alcohol, 0.9 per cent. NaCl, glycerine, and 0.36 per cent. HCl. These were used at various intervals after their preparation and were kept under various conditions of temperature.

In preparing the extracts for use they were filtered, or otherwise separated from the tissue cells, and were then diluted with 3 or 4 volumes of distilled water. The hydrogen ion concentration of the mixture was adjusted to the desired level by adding either 0.36 per cent. HCl or dry sodium carbonate. All tests of enzymatic action reported here must be looked upon as qualitative only.

Action of the Extracts on Protein (Fibrin).

For the experiments on the digestion of protein, fibrin was extracted from fresh blood and was then kept in glycerol. Before use it was thoroughly washed; dried between leaves of blotting paper; and then minced and small portions of it were placed in several test tubes along with the extract to be tested and a few drops of toluene. The whole mixture was incubated at 35° C.

In order to rule out the problem of autolysis the reaction of the media during these tests was adjusted to H ion concentrations of pH = 2; pH = 8.0; pH = 8.4 and pH = 9.0 respectively.

No trace of digestion of fibrin was manifested in periods ranging from 48 to 62 hours respectively, a fact which indicates that the extract of intestinal mucous membrane contains neither pepsin nor trypsin.

In one case, on the sixth day the fibrin was found to be partly dissolved. The initial pH of the mixture in this case was 8.4, but it may have been that during such a long period of incubation the medium may have lost its alkalinity to such an extent that the autolytic enzyme-complexes had started to act.

Action of the Extracts on Peptone.

In one set of experiments a very gradual but not complete discoloration of a mixture of purified casein, phenol red, and saline extract was observed during 24 hours in the incubator. The initial pH of the mixture was 8.0.

In another set of experiments a 2 per cent. solution of Witte's peptone was mixed with boiled, and with unboiled, saline intestinal extract, respectively, and was adjusted to pH = 7.8. After 3 days in the incubator biuret tests showed that there was less peptone in the mixture with fresh extract, than in the control, in which boiled extract was used, indicating that the fresh extract had a weak ereptic action.

Action of the Extracts on Starch.

Table VI. gives the results of two experiments in which the extract of the intestinal mucous membrane on normal saline was allowed to act on starch. In one case fresh extract was used and in the other the extract was boiled. In each case the mixture was

adjusted to pH 6.7 and 2 drops of toluene were added, after which it was placed in the incubator. In the subsequent test for starch a weak solution of iodine in potassium iodide was employed. The table shows that, whereas the tests with boiled extract were negative, those with fresh extract gave a progressive diminution in the amount of starch present and a corresponding increase in the sugar content of the mixture, indicating a decided amylolytic action.

That the amylolytic action of the intestinal extract was due to the enzyme contained in the mucous membrane and not to the pancreatic juice, which could be fixed on the intestinal mucous membrane, in spite of its thorough washing, is seen from the fact that proteolytic action was absent in the same extracts.

TABLE VI.

AMYLOLYTIC ACTION OF EXTRACT OF THE INTESTINAL MUCOUS MEMBRANE.

Date.	Experiment 1.	Experiment 2.
August 4.....	3 cc. of 2% soluble starch 10 drops boiled extract pH = 6.7 Iodine reaction—blue	3 cc. of 2% soluble starch 10 drops fresh extract pH = 6.7 Iodine reaction—blue
3.30 P.M.....	Placed in incubator	Placed in incubator
August 5:		
8.30 A.M.....	Iodine—no change Fehling—negative pH = 6.7	Iodine—light purple Fehling—positive pH = 6.7
7.30 P.M.....	Iodine—no change	Iodine—light purple
August 6:		
10.15 A.M.....	Iodine—no change Fehling—no change pH = 6.6	Iodine—very light purple Fehling—strongly positive pH = 6.7
7.30 P.M.....	—no change	—progressive change
August 7:		
7.30 P M.....	Iodine—no change Fehling—no change pH = 6.6	Iodine—almost colorless Fehling—strongly positive pH = 6.7

Action of the Extracts on Lipoids.

It was found that unboiled extract of the intestinal mucous membrane had a weak lypolytic action on olive oil and a somewhat more pronounced effect on cream. The reaction on cream is shown in Table X., experiments 5 and 6.

Enterokinase of the Extract.

In addition to its action as a ferment acting directly on peptones, starch and fats, it was shown that the unboiled intestinal extract had also the power of greatly accelerating the tryptic action of liver extract of *Fundulus* and of the bile: to a lesser degree, this extract increased the action of lipase on the digestion of fat, whereas boiled extracts had no such effects.

Activation of Trypsinogen.

Table VII. shows the results of adding unboiled saline intestinal extract to a mixture containing minced fibrin and glycerin extract

TABLE VII.

ACTIVATION OF TRYPSINOGEN BY EXTRACT OF INTESTINAL MUCOUS MEMBRANE.

Date.	Exp. 1.	Exp. 2.	Exp. 3.
August 13:			
12.00 Noon	Minced fibrin 8 dr. glycerin extract of hepatic pancreas 12 dr. water No intestinal extract (control) pH = 8.2	Minced fibrin 8 dr. glycerin extract of hepatic pancreas 10 dr. water 2 dr. intestinal extract (in saline) pH = 8.2	Minced fibrin 8 dr. glycerin extract of hepatic pancreas; and intestinal mucous membrane prepared a day before 12 dr. water 2 dr. intestinal extract (in saline) pH = 8.2
1.15 P.M.	No change	No change	Completely digested
3.30 P.M.	" "	Digestion begun	—
5.30 P.M.	" "	Half digested	—
6.30 P.M.	" "	Completely digested	—
August 14:			
9.00 A.M.	" "	—	—
August 15:			
9.00 A.M.	" "	—	—
August 16:			
8.30 A.M.	Marked digestion	—	—
1.00 P.M.	Half digested	—	—
3.00 P.M.	Completely digested	—	—
			N.B. In this experiment (3) the extracts of hepatic pancreas and of intestine were mixed together on the previous day

of the liver of *Fundulus*. (As stated above, the liver of these fishes is invaded by pancreatic tissue, so that extracts might contain considerable trypsinogen.) In Experiment No. 3, the fibrin was very quickly digested. The probable cause was that the liver extract and the extract of the intestine had been mixed together on the previous day and that in the interval the trypsinogen had already become active trypsin. In Experiment No. 2, the activation of protrypsin required a latent period of about 3 hours. The presence of glycerin in the mixture probably inhibited the process of activation. In Experiment No. 1, intestinal extract was not used, and, therefore, digestion did not take place until the third day. The most probable explanation of this phenomenon is that it required such an interval for the spontaneous activation of the protrypsin.

Other experiments which demonstrate the activation of trypsinogen by intestinal extracts are recorded in Table IX.

Acceleration of Lipolysis.

The experiments in which the digestion of fat was accelerated by the addition of unboiled intestinal extract to a mixture containing bile and cream are recorded in Table X. and in Table XI.

Enzymes in Gall-bladder Bile.

At the time of these experiments, the histological investigations had not been made and, therefore, it was not then known that the gall-bladder of *Fundulus* is surrounded by a thin layer of pancreatic tissue, the presence of which introduces the possibility that a small amount of pancreatic enzyme may have become mixed with the bile during the manipulations for emptying the gall-bladder.

The gall-bladder was dissected out and removed from the body with sterile instruments, the bile was pressed out of the viscus, and the contents were collected in a sterile container. The bile secured in this manner contained a very active amylase, trypsinogen and prolipase. The last two substances could be activated by the unboiled extract of intestinal mucous membrane.

Table VIII. gives the results of two experiments to demonstrate the amylolytic action of fresh bile.

Table VIII.

Amylolytic Action of Gall-bladder Bile.

August 7:

Time.	Experiment 1.	Experiment 2.
9.30 A.M.....	2 cc. of 2% soluble starch 2 drops boiled bile pH = 6.6	2 cc. of 2% soluble starch 2 drops fresh bile pH = 6.6
9.40 A.M.....	Placed in water bath at 35° C.	Placed in water bath at 35° C.
10.40 A.M.....	Iodine—dark blue Fehling—negative	Iodine—purple color Fehling—positive
11.40 A.M.....	No change	Iodine—light purple Fehling—strongly positive
2.40 P.M.....	No change pH = 6.6	Iodine—very light purple almost colorless Fehling—strongly positive pH = 6.6

Table IX.

Trypsinogen of the Bile and its Activation by the Extract of the Intestinal Mucous Membrane.

Date and Time.	Experiment 1.	Experiment 2.	Experiment 3.
August 5:	Minced fibrin 2 cc. bile 8 cc. dist. water No extract (control) pH = 8.3	Minced fibrin 2 cc. bile 8 cc. dist. water 1 drop boiled intestinal extract pH = 8.3	Minced fibrin 2 cc. bile 8 cc. dist. water 1 drop fresh intestinal extract pH = 8.2
10.20 A.M.	Put in incubator	Put in incubator	Put in incubator
12.05 P.M.	No change	No change	Digestion begun
1.15 P.M.	" "	" "	Half digested
2.00 P.M.	" "	" "	Completely digested
7.30 P.M.	" "	" "	—
August 6:			
10.20 A.M.	" "	" "	—
	Added 1 drop fresh intestinal extract	Added 1 drop fresh intestinal extract	
11.30 A.M.	Digestion begun	Digestion begun	
12.05 P.M.	Half digested	Half digested	
1.00 P.M.	Completely digested	Completely digested	

Table IX. shows that the bile alone or in the presence of boiled intestinal extract has no digestive action on fibrin, but when fresh

Table X.

Prolipase in Gall-bladder Bile Lipase and Enterokinase in Extracts of Intestinal Mucous Membrane.

Date.	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.	Experiment 5.
Aug. 12.....	Cream substrate (control)	Cream substrate 4 dr. bile	Cream substrate 4 dr. bile 2 dr. fresh intestinal extract	Cream substrate No bile 4 dr. boiled intestinal extract	Cream substrate No bile 4 dr. fresh intestinal extract
3.00 P.M.....	Put in incubator	Put in incubator	Put in incubator	Put in incubator	Put in incubator
4.30 P.M.....	No change	Light purple 1 dr. NaOH added	Light green 2 dr. NaOH added	No change	No change
6.00 P.M.....	" "	Purple	Light green 3 dr. NaOH added	" "	" "
8.00 P.M.....	" "	Light green 1 dr. NaOH added	Light purple 1 dr. NaOH added	" "	Light pink
Aug. 13: 8.00 A.M.....	" "	Light green 3 dr. NaOH added	Grey 2 dr. NaOH added	" "	White 1 dr. NaOH added
3.00 P.M.....	" "	Grey-purple 1 dr. NaOH added	Pink-purple 1 dr. NaOH added	" "	Pink
NaOH solution added during 24 hours	Nil.	6 drops	9 drops	Nil.	1 drop

Table XI.

Prolipase in the Gall-bladder Bile, its Activation by the Intestinal Extract.

Date.	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.	Experiment 5.
Aug. 16.....	Cream substrate (control)	Cream substrate 5 dr. of boiled bile	Cream substrate 3 dr. of fresh bile	Cream substrate 2 dr. boiled bile and 2 dr. boiled intestinal extract	Cream substrate 2 dr. of fresh bile and 2 dr. of fresh intestinal extract
11.00 A.M.....	Put in incubator	Put in incubator	Put in incubator	Put in incubator	Put in incubator
12.00 Noon.....	No change	No change	Light pink 1 dr. NaOH added	No change	Light pink 1 dr. NaOH added
1.00 P.M.....	" "	" "	No change	" "	Light pink 1 dr. NaOH added
2.00 P.M.....	" "	" "	Very little change	" "	Grey 1 dr. NaOH added
5.00 P.M.....	" "	" "	No change	" "	Grey 3 dr. NaOH added
8.30 P.M.....	" "	" "	" "	" "	Grey 3 dr. NaOH added
Aug. 17: 8.00 A.M.....	" "	" "	Grey purple 1 dr. NaOH added	" "	Light pink 2 dr. NaOH added
NaOH solution added during 17 hours	Nil.	Nil.	2 drops	Nil.	11 drops

extract is used, tryptic digestion takes place in a short time. Apparently trypsinogen was present in the bile, and was activated by the fresh intestinal extract.

To these findings might also be added the fact that when toluene is not used, self activation of the tryptic enzyme in the bile takes place after one or two days at room temperature, and much more quickly in the incubator.

Tables X. and XI. show that the gall-bladder bile contains a prolipase, which may be activated by the extract of the intestinal mucous membrane. From the same tables we learn too that the intestinal extract possesses a weak lipolytic action and contains an activator for the bile lipase.

In all of the experiments recorded in Tables X. and XI. boiled cream, diluted with one volume of distilled water was used as the substrate, and to this was added one drop of 1 per cent. phenolphthalein and sufficient 0.09 per cent NaOH solution (2 to 4 drops) to produce in each case a distinct pink color ($pH = 8.3$). This alkaline pink mixture of diluted boiled cream is referred to in the table as the cream substrate. After decoloration of the mixture in the incubator, a sufficient amount of 0.09 per cent. NaOH solution was added to restore its initial pink color.

From Tables X. and XI. one may see that: the bile alone possesses a weak lypolitic action which develops slowly (Exp. 2, Table X. and Exp. 3, Table XI.); this action is due to an enzyme, because boiled bile loses its lypolitic action (Exp. 2, Table XI.); the intestinal extract alone has a weak lipolytic action (Exp. 5, Table X.); boiled intestinal extract loses its enzymatic action (Exp. 4, Table X.); when bile and intestinal extract are combined the lipolysis is much accelerated (Exp. 3, Table X. and Exp. 5, Table XI.); boiling destroys the lipolytic action and the activation (Exp. 4, Table XI.).

It is evident that the result of combined action of the bile and of the intestinal extract is not merely a simple summation of their activities, but indicates rather that the intestinal extract possesses a substance which activates the bile viz. pancreatic lipase. There is also a possibility that the bile increased the action of the intestinal lipase.

Since the presence of pancreatic tissue on the gall-bladder makes

it possible that enzymes of the pancreas may have become mixed with the bile used in these experiments, the latter must necessarily be repeated with bile obtained by more adequate methods. If it can be demonstrated that the enzymes in question were admixed to the bile with the pancreatic cells surrounding the gall-bladder in *Fundulus,* these experiments will offer a proof that the pancreas of this animal elaborates the same enzymes as the pancreas of warm-blooded animals, two of them in form of zymogen (protrypsin and prolipase), and one in active form (amylase).

Discussion.

The data reported in this investigation show that *Fundulus heteroclitus* lacks a stomach, the intestine joining directly to the œsophagus. Since there are several species of fishes with an analogous structure of the alimentary canal, from the point of view of their digestive system, all fishes may be divided into two main groups, namely, (1) those possessing a pepsin-hydrochloric acid digestion, and (2) those lacking it. This makes it possible to differentiate two types of digestion in fishes; namely, acid-alkaline, and exclusively alkaline. There is very little doubt that these decidedly different types of digestion greatly affect the internal chemisms of the body, in the two cases; because, in the one case, important changes in the chemical composition of the body fluids are provoked following the secretion of acid into the stomach. Similar changes must necessarily be quite absent in fishes deprived of a stomach and must be greatly attenuated in the pathological syndrome, known as achylia gastrica, or in animals with experimentally removed stomachs. Therefore, the study of the animals of a type of *Fundulus* presents an interesting problem.

Summary.

1. That *Fundulus heteroclitus* does not possess a stomach is shown by the following evidence:

(*a*) There is absolute absence of pepsin and hydrochloric acid in the digestive juices.

(*b*) Every phase of digestion takes place in an alkaline medium.

(*c*) The first part of the intestine (duodenum) is joined directly to the œsophagus.

(*d*) The bile and pancreatic juice are poured into the gut only a few millimeters (3 or 4) below the œsophagus.

(*e*) The duodenum is capable of dilating to form a container for food.

2. Extracts of the intestinal mucous membrane manifest an amylolytic, a lipolytic and an ereptic digestive action. They probably contain also an enterokinase.

3. The gall-bladder bile contains a trypsinogen, a prolipase and an amylase. The origin of these enzymes must be investigated further, because they could be admixed to the bile from the layer of pancreatic tissue surrounding the gall-bladder, during its collection.

REFERENCES.

1. **Jordan, D. S., and Evermann, B. W.** Bull. of the U. S. Nat. Mus., Washington, 1896, Part 1, p. 640.
2. **Brunning, C.** Wochenschr. Aquar.—Terrar. Kunde 1910, Jg. 7, p. 161.
3. **Gill, T. N.** Proc. U. S. Nat. Mus., 1895, Vol. 17, p. 115; and Ibidem, 1896, Vol. 18, p. 221.
4. **Felton, L. D.** Journ. Biol. Chem., 1921, Vol. 46, p. 229.
5. **Clark, W. M.** "The Determination of Hydrogen Ions," 2d ed. Baltimore, 1922, p. 118.
6. **Bradley, H. C.** Journ. Biol. Chem., 1922, Vol. 52, p. 467.
7. **McFarlane, J., et al.** Journ. Gen. Physiol., 1927, Vol. 10, p. 437.

The authors wish to acknowledge their indebtedness to the Biological Board of Canada for the permission to use the facilities of the Atlantic Biological Station, St. Andrews, N. B., and to Professor A. G. Huntsman and his staff for valuable assistance rendered in the course of the study.